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Persistence of scrapie infectivity within a farm environment after cleaning and decontamination

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Article Summary:

Article focus

- Scrapie infectivity in sheep and goats is widely disseminated from infected animals into their immediate environment, and is responsible for horizontal transmission of disease in the absence of animal to animal contact
- Using a sheep bioassay on a farm affected by scrapie, this study focused on the effectiveness of treatment conditions that are recommended for the decontamination of farm premises following outbreaks of scrapie in sheep and goats
- The study also sought to demonstrate improved methods for the decontamination of scrapie premises.

Key messages

- Recommended decontamination conditions using a 20,000 part per million hypochlorite solution were not effective in removing scrapie infectivity from a barn on a scrapie contaminated farm.
- Complete painting of a pen with replacement of all metalwork, delayed the onset of clinical symptoms but did not stop transmission of scrapie from the environment.
- Scrapie infectivity is very difficult to remove from the environment and current methods for its removal are not effective.

Strengths and limitations of study

- This study is the first of its kind to look at the decontamination of scrapie infectivity from the environment within a scrapie infected farm.
- The study uses a natural transmission model of scrapie, whereby newborn unexposed lambs become exposed to scrapie from their environment alone, rather than a laboratory simulation.

- Whilst scrapie infectivity could have been brought into the decontaminated barn, via vermin or farm workers, high levels of biosecurity throughout the experiment were maintained to reduce other sources of infectivity reaching the penned animals.

Abstract:

Scrapie of sheep/goats and Chronic Wasting Disease (CWD) of deer/elk are contagious prion diseases where environmental reservoirs are directly implicated in the transmission of disease. In this study the effectiveness of recommended scrapie farm decontamination regimes was evaluated by a sheep bioassay using buildings naturally contaminated with scrapie. Pens within a farm building were treated with either 20,000ppm free chorine solution for one hour, or were treated to same but were followed with painting and full re-galvanisation or replacement of metalwork within the pen. Scrapie susceptible lambs of the *PRNP* genotype VRQ/VRQ were reared within these pens and their scrapie status was monitored by RAMALT. All animals became infected over an 18-month period, even in the pen that had been subject to the most stringent decontamination process. This data suggests that recommended current guidelines for the decontamination of farm buildings following outbreaks of scrapie do little to reduce the titre of infectious scrapie material and that environmental re-contamination could also be an issue associated with these premises.

Introduction:

Transmissible Spongiform Encephalopathies (TSEs) are a group of fatal neurodegenerative diseases for which there is no effective treatments or cure. Examples of TSE infections affecting mammalian species include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in deer and elk, and variant CJD (vCJD) and Kuru in man. In each case the etiological agent is proposed to be a conformational isomer (PrP^{Sc}) of the host encoded prion protein (PrP^{C}) (Prusiner 1998). During a prolonged preclinical phase of disease progression host PrP^{C} is converted into PrP^{Sc} which accumulates, particularly in the central nervous system. The conversion of PrP^{C} to PrP^{Sc} confers several changes in the biochemical properties of the protein, such as a decreased solubility in detergents, and an increase in resistance to proteases and chemical denaturants.

For scrapie and CWD, infectious prions are shed from animals via multiple routes during both the preclinical and clinical stages of disease. For example, sheep infected with the scrapie prion secrete/excrete prion within faeces (Terry and others 2011), saliva (Maddison and others 2010b, Gough and others 2012), urine (Rubenstein and others 2011) and skin (Thomzig and others 2007). Furthermore, parturient material is known to harbour high levels of scrapie infectivity (Pattison and others 1972), and its presence correlates with an increase in the transmission of scrapie during the lambing season (Touzeau and others 2006). For scrapie and CWD, the dissemination of PrP^{Sc} coupled with its high stability leads to environmental reservoirs of infectivity. For example it is known that premises that have housed scrapie-infected animals remain a potential source of infectivity for many years (Georgsson and others 2006) and we have demonstrated that scrapie prions can be detected on a range of surfaces within the farm providing likely sources of prion exposure (Maddison and others 2010a). Here, we examine the effectiveness of the recommended decontamination method for farm buildings, the use of 20,000ppm free chlorine, in sodium hypochlorite solution, for one hour. We demonstrate that on

premises that are affected with sheep scrapie, pens cannot be effectively decontaminated either using the recommended decontamination treatments, or using a much more stringent treatment consisting of a complete replacement/re-galvanisation of all metalwork and a complete painting of the pen. Such observations have important implications for the decontamination and restocking of farms following outbreaks of scrapie, and especially in the case of goats, with their lack of scrapie resistant genotypes.

Materials and Methods:

Scrapie affected farm

This study was conducted on an experimental farm with a high incidence of naturally transmitted scrapie. The AHVLA Ripley flock was started in 1998 by the purchase of sheep from flocks with cases of scrapie. In the intervening years this flock has contained up to 350 breeding ewes of a mixture of breeds and genotypes of predominantly VRQ, ARQ, AHQ and ARR alleles. The breeding policy was to maintain a wide range of susceptible genotypes within the flock and animals were reared in line with common practice within British lowland sheep flocks (Ryder and others 2004). Lambing had been carried out within barns each spring where ewes and lambs were kept for up to a week before going to pasture. The study was carried out within a barn that since 2001 had housed scrapie exposed sheep at various stages of scrapie disease progression and been used for lambing, blood sampling and semen collection from animals prior to this study. During this study the barn did not house any sheep other than those used within the described bioassay for the duration of the experiment. The distances between the four pens were exactly the same. With the barn being a standard livestock building there was natural air circulation i.e hit and miss boarding from shoulder height & wind break material at either end of the barn, therefore all pens received the same air flow. The bio-security to all pens was the same during the experiment. During this time the rest of the farm was maintained as a sheep farm with restricted access and which continued the maintenance of a flock of around 100 sheep, which were kept at pasture away from the experimental barn. Animals were occasionally brought in from the field into other buildings on the farm for sampling purposes. Harvesting and other field work were not carried out within the duration of this experiment.

Decontamination methods

A barn was used containing four pens (at the four corners of the barn) each separated from each other by a minimum of 4 metres. Each pen measured 4m x 6.4m. The pens (wall, floor, metalwork) and the pen furniture (hay racks and water trough) were all thoroughly decontaminated during the pen treatments. One pen was left untreated except for brushing out gross debris (pen A). The three other pens were initially pressure washed to remove gross debris (pen B was left just power washed), two were then treated with sodium hypochlorite solution containing 20,000 ppm free chlorine for 1 hour before washing with water in accordance with best practice for inactivating surface contaminating prions (pen C). One of the hypochlorite treated pens then had all moveable metalwork replaced or treated by re-galvanisation, the floor, wall (up to a height of 1.35m) and every item of immovable steel (gate posts) were then painted in a hard wearing floor paint (Pen D). Each pen was accessed via a different entrance leading to a different changing area for each pen with dedicated new clothing, boots and equipment for each pen which were used by all personnel entering the pens to tend the animals (Ryder and others

2009). These changing areas were within the pen decontamination areas, but outside the pen housing the sheep. Fresh feed and bedding was brought onto the farm as required from a scrapie free farm. At delivery feed and bedding were transported directly to each pen treatment area for each pen and stored in new dedicated bins, within each decontaminated area.

Sheep Bioassay:
All procedures were carried out in accordance with the Animal (Scientific Procedures) Act (ASPA) 1986, under licences from the UK Government Home Office. The study was reviewed and approved by the Animal Health and Veterinary Laboratories Agency Ethical Committee. The study was carried out in facilities licenced under ASPA and owned and managed by AHVLA, an Agency of the Department of Food and Rural Affairs (DEFRA).

Lambs originated from the AHVLA scrapie free sheep flock, derived from animals originally imported from New Zealand and maintained in the UK under high levels of biosecurity and which never had any instances of classical scrapie. Three day old VRQ/VRQ lambs from this flock were introduced under tight biosecurity conditions to the 4 pens in groups of 5, 7 days after the decontamination had been completed. Sheep were fed on a diet of Store lamb finisher (Atlees), and hay ad libitum. From 6 months of age recto-anal mucosa associated lymphoid tissue (RAMALT) was taken every 3 months and PrP^{Sc} detected by immunohistochemistry as previously described (Gonzalez and others 2005). Animals that were diagnosed as having preclinical scrapie were removed from the pens and put out into pasture. Sheep remained on pasture until the onset of the clinical signs of scrapie.

Results:
A sheep bioassay was used to determine the extent to which contaminating scrapie prions remain after decontamination steps have been taken to remove them from a naturally contaminated farm building. Pens were either untreated (pen A), power washed (pen B), power washed followed by treatment with 20,000 ppm free chlorine for 1 hour (pen C) or power washed, hypochlorite treated and then all surfaces either replaced, re-galvanised or painted (pen D). A bioassay was then carried out by introducing scrapie susceptible lambs into the decontaminated pens and monitoring them by RAMALT testing every 3 months from 6 months of age (figure 1). There was very little difference in the rate at which animals became infected when comparing the untreated pen (pen A) to those that had been power washed or hypochlorite treated. Remarkably, despite the pen D decontamination regime consisting of a complete repaint of every surface and replacement or re-galvanisation of all metalwork, all five sheep were also scrapie-positive by 18 months of age. This data clearly show that despite an exceptionally stringent pen cleaning regime, there is still enough prion agent within the pen for the infection of sheep. The slower kinetics of the sheep becoming both RAMALT positive, and a longer average time until observation of clinical symptoms in pen D compared to pens B and C are consistent with a lower infectious dose in pen D after the decontamination regime.

Discussion:

Thorough pressure washing of a pen had no effect on the amount of bio-available scrapie infectivity (pen B). The routine removal of prions from surfaces within a laboratory setting is treatment for a minimum of 1 hour with 20,000 ppm free chlorine; a method originally based on the use of brain macerates from infected rodents to evaluate the effectiveness of decontamination (Kimberlin and others 1983). Further studies have also investigated the effectiveness of hypochlorite disinfection of metal surfaces to simulate the decontamination of surgical devices within a hospital setting. Such treatments with hypochlorite solution were able to reduce infectivity by 5.5 logs to lower than the sensitivity of the bioassay used (Lemmer and others 2004). Analogous treatment of the pen surfaces did not effectively remove the levels of scrapie infectivity over that of the control pens, indicating that this method of decontamination is not effective within a farm setting. This may be due to the high level of biological matrix that is present upon surfaces within the farm environment, which may reduce the amount of free chlorine available to inactivate any infectious prion. Remarkably 1/5 sheep introduced into pen D had also became scrapie positive within 9 months, with all animals in this pen being RAMALT positive by 18 months of age. Pen D was no further away from the control pen (pen A) than any of the other pens within this barn. Localized hot spots of infectivity may be present within scrapie contaminated environments, but it is unlikely that PenD area had an amount of scrapie contamination that was significantly different than the other areas within this building. Similarly there were no differences in how the biosecurity of PenD was maintained, or how this pen was ventilated compared to the other pens. This observation perhaps indicates the slower kinetics of disease uptake within this pen and is consistent with a more thorough prion removal and recontamination. These observations may also account for the presence of inadvertent scrapie cases within other studies, where despite stringent biosecurity, control animals have become scrapie positive during challenge studies using barns that also housed scrapie affected animals (Ryder and others 2009). The bioassay data indicates that the exposure of the sheep to a farm environment after decontamination efforts thought to be effective in removing scrapie is sufficient for the animals to become infected with scrapie. The main exposure routes within this scenario are likely to be via the oral route, during feeding and drinking, and respiratory and conjunctival routes. It has been demonstrated that scrapie infectivity can be efficiently transmitted via the nasal route in sheep (Hamir and others 2008), as is the case for CWD in both murine models and in white tailed deer (Denkers and others 2010, 2013). Recently it has also been demonstrated that CWD prions presented as dust when bound to the soil mineral Montmorillonite can be infectious via the nasal route (Nichols and others 2013). When considering pens C and D, the actual source of the infectious agent in the pens is not known, it is possible that biologically relevant levels of prion survive on surfaces during the decontamination regime (pen C). With the use of galvanizing and painting (Pen D) covering and sealing the surface of the pen, it is possible that scrapie material re-contaminated the pens by the movement of infectious prions contained within dusts originating from other parts of the barn that were not decontaminated or from other areas of the farm..

Given that scrapie prions are widespread on the surfaces of affected farms (10), whatever the source of the infectious prions in the pens this study clearly highlights the difficulties that are faced with the effective removal of environmentally associated scrapie infectivity. This is likely to be paralleled in CWD which shows strong similarities to scrapie in terms of both the dissemination of prions into the environment as well as the facile mode of disease transmission. These data further contribute to the understanding that prion diseases can be highly transmissible

between susceptible individuals not just by direct contact but through highly stable environmental reservoirs that are refractory to decontamination.

The presence of these environmentally associated prions in farm buildings make the control of these diseases a considerable challenge, especially in animal species such as goats where there is lack of genetic resistance to scrapie and therefore no scope to re-stock farms with animals that are resistant to scrapie.

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Figure Legends

Fig. 1. Transmission of environmentally acquired scrapie as monitored by RAMALT. Lambs were housed in four separate pens that had each been treated using four different decontamination regimes. Pens were either untreated (pen A), power washed (pen B), power washed followed by treatment with 20,000 ppm free chlorine for 1 hour (hypochlorite treated; pen C) or power washed, hypochlorite treated and then all surfaces either replaced, re-galvanised or painted (pen D). The five sheep in each pen were monitored every 3 months from 6 months of age. All sheep in each of the 4 pens were RAMALT positive by 18 months of age. *indicates the mean incubation period for each pen before clinical signs were observed.